FOREWORD

Concerning Supplements to

The Journal of Nutrition

To meet the need for publication of meritorious but unusually long manuscripts the Journal of Nutrition instituted in April 1954 the policy of publishing such papers in the form of supplements to regular issues. The authors provide the full cost of such publication. For a more complete statement regarding this policy see volume 52, Supplement 1, April, 1954.

GEORGE R. COWGILL, *Editor*

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TRYPTOPHAN-NIACIN RELATIONSHIPS IN MAN

STUDIES WITH DIETS DEFICIENT IN RIBOFLAVIN AND NIACIN, TOGETHER WITH OBSERVATIONS ON THE EXCRETION OF NITROGEN AND NIACIN METABOLITES

By

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STUDIES WITH DIETS DEFICIENT IN RIBOFLAVIN AND NIACIN, TOGETHER WITH OBSERVATIONS ON THE EXCRETION OF NITROGEN AND NIACIN METABOLITES [1]

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Although the prevalence of pellagra in areas where maize formed a considerable portion of the diet had been observed for several centuries, it is only in recent years that it has been possible to attempt to define the relative roles of niacin (Smith, '42), riboflavin (Sebrell and Butler, '38; Horwitt et al., '49b; Hills et al., '51) and tryptophan (Krehl et al., '46; Elvehjem, '48 and Goldsmith et al., '52) in the etiology of this disease. From these studies it would appear that the association of corn products with pellagra is more a function of the low tryptophan content of corn than of any "toxin" (Woolley, '46). The possibility remains that some links in this puzzle may still be missing.

[1] This project was supported by grants from the National Vitamin Foundation, Inc., and was sponsored by the Committee on Vitamin Studies at Elgin State Hospital, Food and Nutrition Board, National Research Council. During the period of this study, the committee was constituted as follows: Dr. Russell M. Wilder, chairman; Dr. O. A. Bessey, Dr. E. S. G. Barron, Dr. R. S. Goodhart, Dr. M. K. Horwitt, Dr. A. C. Ivy, Dr. R. M. Kark, Mr. Park Livingston, Dr. Erich Liebert, Dr. A. A. Lieberman, Dr. D. L. Steinberg and Dr. Phyllis Wittman.

[2] U. S. Public Health Service Fellow, 1950–51. Now at Mayo Clinic, Rochester, Minn. Dr. H. H. Hiatt was a U. S. Public Health Service Fellow on this project from June, 1951 to March, 1952, during which period he made important contributions.

This report will describe that part of Elgin National Research Council Project no. 3 which dealt with attempts to evaluate man's needs for niacin and tryptophan in diets low in corn products and both adequate and deficient in riboflavin. In order to decrease the level of tryptophan in the basal diets without using large amounts of corn meal, it was necessary to keep the protein intake at a low level. As a consequence of either this low protein level or of some other factor, changes in liver functions were observed after 5 months. These observations of liver dysfunction in man, produced and ameliorated by varying protein intake (Horwitt, '53; Horwitt, Rothwell and Kark, '53; Kark et al., '53) and the associated alterations in carbohydrate metabolism, will be discussed elsewhere, as these effects had no apparent direct relationship to niacin requirements although the implications of liver dysfunction did necessitate alterations in the original plan of the project. Though the liver dysfunction might possibly influence the rate at which the metabolites studied appeared in the urine in a short-term sample, such effects could not be noted in the 24- or 72-hour urine samples studied. Therefore, the data reported in this paper are considered to be similar to those which might be obtained from healthy subjects.

METHODS OF STUDY

As in previous long-term nutritional studies at Elgin (Horwitt et al., '48), the selection of subjects was made after evaluating the physical and mental qualities of a large number of mental patients. Choice was based upon chronicity of mental illness, adequacy of physical condition, and the presence of a reasonable amount of emotional stability. After a three-month trial period during which attitudes, cooperativeness and appetite habits were observed, 40 male subjects were divided into 5 experimental groups. The subjects were relatively young. All but one were 30 years or over, 8 were over 45 years and only three were over 60 years at the start of the study.

Diets, dietary supplements and division of subjects

The organization of the diet kitchen and the techniques used to control the dietary intake were similar to those previously described (Horwitt et al., '49c). The three basal diets [3] used are given in table 1. Three different sets of recipes were available so that, in effect, 9 different daily menus were rotated in an effort to provide some variety. A sample of the diet was homogenized and analyzed daily for nitrogen. Other constituents were determined at frequent intervals. The basal diets provided approximately 2300 Cal., 6.5 gm of protein nitrogen, 86 gm of fat, 5.8 mg of niacin, 265 mg of tryptophan and 415 μg of riboflavin.

As the quality of the protein in the diet may be of greater significance than the amount, it is pertinent to point out that the average amounts from wheat flour, zein [4] and gelatin used in the three basic diets were 15.1, 6.0 and 3.9 gm per day, respectively. It should also be noted that, since these diets contained approximately 41 gm of protein (6.59 gm of protein nitrogen) per day, they provided about 15 gm less protein than the diets of the previous Elgin studies. The 6.0 gm of zein and the 3.9 gm of gelatin were included as part of the total allotment in order to decrease further the tryptophan content of the diet. All of the above-mentioned data were calculated on the basis of 100% consumption of the diet. Actually, two subjects were allotted 90%, 21 subjects received 100%, three subjects received 110% and 5 subjects received 120% of all the ingredients of the basal diet in accordance with their appetite, size and personal preference as determined during the preliminary period. During the experimental period the percentage of the allotted food not consumed by any subject was negligible (less than 2%).

All subjects on the controlled diets received a daily vitamin supplement which contained 30 mg of ascorbic acid,

[3] Feeding of the basal diet began November 6, 1950.

[4] Miss Alice Hay, who served as dietitian during the early stages of this work, demonstrated considerable ingenuity in devising tea rolls and doughnuts which combined zein and flour in proportions that were palatable.

TABLE 1

Constituents of daily diets used in Elgin project no. 3

FOOD	SOURCE	WT.	DIET 1				DIET 2				DIET 3			
			Protein	Tryptophan	Niacin	Riboflavin	Protein	Tryptophan	Niacin	Riboflavin	Protein	Tryptophan	Niacin	Riboflavin
		gm	*gm*	*mg*	*μg*	*μg*	*gm*	*mg*	*μg*	*μg*	*gm*	*mg*	*μg*	*μg*
Salt pork	Purchased	25	1.0	12	225	10	1.0	12	225	10	1.0	12	225	10
Farina [1]	Special	30	3.3	36	240	18								
Cornflakes [1]	Special	30					2.4	12	480	30	2.4	12	480	30
Pears	Home canned	100	0.2	2	100	20								
Pears	Home canned	50									0.1	1	50	10
Applesauce	Dried apples	100	0.4		200	20	0.4		200	20	0.4		200	20
Bread [2]	Special	120	8.6	70	1080	131	8.6	70	1080	131	8.6	70	1080	131
Margarine	A.P.	40	0.2				0.2				0.2			
Jelly	Homemade	60	0.1		120	12	0.1		120	12	0.1		120	12
Beef	Round [3]	25	4.9	69	1175	42	4.9	69	1175	42	4.9	69	1175	42
Potatoes	Varied	100	2.0	16	1200	40					2.0	16	1200	40
Spaghetti [4]	A.P.	50					6.4	51	1000	30				
Rice	Boiled	30	2.3	24	480	9	2.3	24	480	9	2.3	24	480	9
Cookies	Special	67	2.6	24	209	12								
Cabbage	Varied	50					0.7	7	150	25				
Sauerkraut	Homemade	50									0.6	6	50	30
Pudding	Vanilla	100					0.1							
Cream, 40%	A.P.	25	0.6	5	25	28	0.6	5	25	28	0.6	5	25	28
Sugar [5]														
Cake	Special	75									3.2	26	266	15
Tomatoes	Home canned	50	0.5	5	350	15	0.5	5	350	15				
Jello	34%	34	3.9				3.9				3.9			
Zein crackers	Special	55	9.9	33	270	15								
Zein rolls	Special	46					7.8	20	151	8				
Zein doughnuts	Special	106									9.6	38	341	19
Rhubarb	Home canned	100					0.4	4	100					
Dill pickles	Canned	25									0.2	2		15
Total (calculated) [6]			40.5	296	5674	372	40.3	290	5536	361	40.1	281	5692	411
Total (as analyzed)			40.6 [7]	274	5790	413	40.0	251	5570	422	41.1	268	5910	408
			±2.5	± 20	± 630	± 37	± 2.8	± 8	± 360	± 35	± 2.2	± 17	± 310	± 34

[1] Prepared on special order by Quaker Oats Company.
[2] Baked from unenriched flour.
[3] Diets 1 and 3: round roast; diet 2: round ground.
[4] Unenriched, as purchased.
[5] Diets 1 and 2: 20 gm; diet 3: 10 gm.
[6] Agricultural Handbook no. 8.
[7] Grams nitrogen multiplied by 6.25.

0.6 mg of thiamine, 1.0 mg of pyridoxine, 3.0 mg of calcium pantothenate, 0.001 mg of vitamin B_{12}, 0.1 mg of folic acid, 0.05 mg of biotin, 4000 I.U. of vitamin A and 400 I.U. of vitamin D.[5] In addition, tricalcium phosphate, 0.5 gm, and ferrous sulfate, 0.25 gm were given three times a week.

At the end of the three-month preliminary period, during which time base line data were accumulated, an attempt was made to distribute the 40 subjects uniformly with respect to age, weight, and psychological state, into the following 5 groups:

1. U group (unsupplemented). Eight subjects received the basal diet (one 90%, 6 100%, and one 120%) plus 0.1 mg of riboflavin per day; this small addition of riboflavin raised the intake of this vitamin to the level used in Elgin Project no. 2 (Horwitt et al., '49c). (After 38 weeks, in order to repair the ariboflavinosis which had developed, this group was supplemented with additional riboflavin, so that in effect, this group was thereafter equated with the "R" group.)

2. R group (riboflavin supplemented). Eight subjects received the basal diet (five 100%, two 110% and one 120%) plus 2 mg of riboflavin, daily.

3. NR group (niacinamide and riboflavin supplemented). Seven subjects received the basal diet (four 100% and three 120%) plus 2 mg of riboflavin and 10 mg of niacinamide, daily.

4. TR group (tryptophan and riboflavin supplemented). Eight subjects received the basal diet (one 90%, 6 100% and one 110%) plus 2 mg of riboflavin and 50 mg of L-tryptophan daily. The tryptophan supplement was doubled to 100 mg after 10 weeks.

5. HD group (Hospital diet). Nine subjects partook of the general hospital diet ad libitum, and were simultaneously subjected to the various procedures applied to the patients on the experimental diet. As a precaution they received a commercial vitamin mixture[6] three times a week which provided ap-

[5] This special formula prepared by Hoffmann-La Roche Inc., through the courtesy of Dr. E. L. Sevringhaus and Dr. M. D. Schiffrin.

[6] Vi-Penta capsules.

proximately 4 mg of niacinamide per day beyond that in the regular diet. The vitamin supplementation for this group was discontinued at the 38th week.

In former Elgin projects, it was relatively simple to maintain a given research plan similar to the one described above. In the present study only the first 37 weeks might be considered to have proceeded according to plan. The problem of liver dysfunction which developed after 5 months on the low-protein diet and the relative urgency of obtaining data which might be related to kwashiorkor made it expedient to alter the original goal somewhat in the hope that liver dysfunction might be proved and repaired without vitiating later interpretation of the data on niacin requirements. The following changes in the dietary supplements were made during the course of the experiment:

22nd to 38th week: An additional 0.5 mg of thiamine was given to half of the subjects in the U, R, NR and TR groups. Above average increases in blood lactic and pyruvic acids had been obtained after glucose ingestion and, although previous experience had indicated that the level of thiamine provided (approximately 0.9 mg per day) was not responsible for this metabolic change and that it was probably a manifestation of liver dysfunction, it was considered wise to supplement half the experimental subjects with thiamine for about 4 months in order to eliminate any doubt on this point. Further addition of 5 mg of thiamine, twice daily, was later tested on three subjects who had a marked disturbance of lactate and pyruvate metabolism (61st to 65th week) but increases in blood lactic and pyruvic acids were not reversed until after 30 gm of lactalbumin was given as a supplement, or a diet high in protein foods was fed.

39th to 61st weeks: lactalbumin, 10 gm daily, was administered to one-third of the subjects in the U, R, NR and TR groups, hereafter called subgroup I.

39th to 52nd weeks: L-lysine, 500 mg twice daily, was administered to another one-third of subjects, hereafter called

subgroup II; DL-methionine, 165 mg twice daily, administered to final third of subjects, hereafter called subgroup III.

52nd to 61st week: zein in basal diet of subgroup II was replaced by equivalent amount of lactalbumin, 7 gm daily, to check possible "toxic effect" of zein.

52nd to 61st weeks: choline dihydrogen citrate, 0.65 gm twice daily, was given to subjects in subgroup III to replace methionine.

52nd week: two subjects, one from TR and one from NR group, each with high bromsulfalein retention, were placed on the HD diet with added animal protein. Decrease in BSP retention level obtained (Horwitt, '53).

61st to 88th week: lactalbumin, 30 mg daily was administered to all remaining subjects (18) from U, R, NR and TR groups who had given evidence suggestive of hepatic dysfunction or of impaired lactic and pyruvic acid metabolism.

61st to 89th week: folic acid, calcium pantothenate, pyridoxine and vitamin B_{12} were removed from the vitamin supplements of the 6 subjects with no apparent aberration of either liver function or of lactic acid and pyruvic acid metabolism. This step was taken in an effort to determine whether these vitamins were preventing or delaying the development of a pellagra syndrome.

88th week: all subjects were placed on extra meat plus regular hospital diet similar to that which had been given to the original 9 subjects in the HD group.

Although most of the subjects were maintained in the experimental unit for the duration of the study, the following separations from the original groups did occur:

Subject U3, 42nd week, was transferred to HD group following appendectomy for acute appendicitis with perforation.

Subject TR5, 66th week, was placed on high-protein, low-calorie diet because of increasing obesity and high glucose tolerance curve. As a consequence of psychotic behavior, subjects U2 and U4 were transferred from their experimental diet to the hospital diet in 69th week. Subjects U8, R1 and TR2

were discharged from unit in 69th, 69th and 38th week, respectively, for unrelated clinical reasons.

The above separations are not considered to be directly related to the experimental procedures but are more a function of normal turnover. The fact that several of the separations came from the "U" group is probably not significant, as their behavior was consistent with their pre-experimental records. There was some question about the accuracy of the food intake of subject R7-110. Consequently, data from this individual have been omitted from the final tabulations.

Tests employed

Some of the procedures used were:

Nitrogen. A complete day's diet (100%) was acidified with acetic acid and homogenized. Samples of the homogenate were analyzed for nitrogen by a modification of the semi-micro method of Redemann ('39). Aliquots of the same homogenate were used for the other dietary assays listed below. The analyses of nitrogen in the urine were made on 72-hour collections. These three-day samples were acidified, cooled or frozen, depending upon when they were to be analyzed and used for all the urine analyses listed.

Fat in the diet. This was estimated by extraction with a 3 to 1 alcohol-ether mixture. The portion of the dried extract soluble in petroleum ether was considered "fat."

Riboflavin. The riboflavin in the diet and in an acidified aliquot of the 72-hour urine sample was analyzed by the microbiological technique of Roberts and Snell ('46). Riboflavin in the red blood cells and plasma was determined by the method of Burch et al. ('48) and will be discussed in a separate communication.

Nicotinic acid. The microbiological method used was similar to the U.S.P. modification of the method of Snell and Wright ('41). Because of the high fat content of the diet it was necessary first to extract most of the lipids with an alcohol-ether mixture.

Quinolinic acid. The method of Henderson ('49) (decarboxylation with glacial acetic acid) was used to treat the urine in order to convert quinolinic acid to nicotinic acid. The total niacin was then determined by the method described above and the quinolinic acid estimated as the difference between the free nicotinic acid in the urine and the total obtained after decarboxylation.

N^1-Methylnicotinamide (N^1-Me). Urinary N^1-Me was determined by the method described by Carpenter and Kodicek ('50).

Xanthurenic acid. The amount in urine was estimated by the technique of Porter et al. ('47, '48).

6-Pyridone of N^1-methylnicotinamide (6-pyr). The amounts in urine were estimated by the methods of Holman and de Lange ('49) and Rosen et al. ('49).

Tryptophan. Diets were dried and defatted. Samples for tryptophan assay were hydrolyzed enzymatically by the method of Greenhut et al. ('46). The amounts of tryptophan in the diets and urine were determined by using the microbiological assay method of Greene and Black ('44), using *L. arabinosus.* Some of the urine samples were also assayed using *S. faecalis* and the medium of Henderson and Snell ('48).

Lysine. Defatted diet samples were analyzed for lysine by the microbiological method of Steele et al. ('49). A similar technique was used for estimation of lysine in the urine. Current chromatographic techniques were also employed as qualitative confirmations.

Creatinine. The amount in urine was estimated by the technique of Bonsnes and Taussky ('45).

Glucose, pyruvic acid and lactic acid in blood. The methods used for the estimation of these compounds and the technique of combining a glucose tolerance test with the simultaneous administration of mild exercise have been described by Horwitt and Kreisler ('49).

Bromsulfalein retention time. The percentage of bromsulfalein remaining 45 minutes after injection of 5 mg per kilo was determined photometrically.[7]

Eosinophils. The extent of eosinopenia produced by adrenocorticotropic hormone (ACTH) was determined by taking an eosinophil count on venous blood before and 4 hours following intramuscular injections of 25 mg of ACTH according to the method of Thorn et al. ('48). Results reported by Hiatt et al. ('52).

Packed cell volume, albumin/globulin ratios, hemoglobin, erythrocyte counts, differential counts, gastric hydrochloric acid, serum cholesterol, N.P.N. and urinary sediments. Determined quantitatively by standard techniques described in Hepler ('49).

Urine was analyzed qualitatively for pathological constituents, such as hemoglobin, albumin and glucose.

The *clinical tests* used included frequent examinations of the skin, oral cavity, cardiovascular system, metabolic rate, blood pressure, pulse rate, and gastrointestinal and liver functions. An attempt was made to evaluate the effects of ultraviolet and infra-red light exposure. The latter experiments were motivated by the findings of Smith and Ruffin ('37) that typical dermatitis could be produced in a pellagrin by exposure to heat as well as sunlight. The subjects were weighed before bedtime every Saturday and more often when a weight change was suspected.

OBSERVATIONS

No change in mental status was observed beyond the variations which might be anticipated in two years (preliminary plus experimental periods) in a group of patients with dementia praecox. There was no apparent diminution in appetite or change in bowel function (patients' complaints were practically non-existent). During the 35th week a subject in the "U" group (riboflavin- and niacin-deficient) with a rather

[7] These were read by Miss D. Rix in Dr. R. M. Kark's laboratory at the University of Illinois in order to facilitate comparison with other liver function tests being conducted simultaneously.

marked erythema of the oral mucus membranes complained casually of a "soreness of the mouth." No other subjective complaints could be evoked even from those subjects of the "U" group who had marked oral pathology or scrotal dermatitis. A few of the subjects had mild upper respiratory infections from which they recovered without apparent sequelae.

Ariboflavinosis

The striking changes noted in the subjects on the low-riboflavin regimen completely confirmed those noted in the previous study (Horwitt et al., '49b) during which the diet provided an equivalent amount of riboflavin (0.55 mg per day), more protein (57 gm), a supplement of 10 mg of niacinamide per day, but did not provide a vitamin B_{12} supplement. Of the 8 subjects in the "U" group, 4 developed diffuse erythema of the buccal and palatal mucosa and a "beefy" redness (but no magenta color) of the tongue with little or no papillary atrophy, within 27 to 36 weeks after the institution of the experimental diet. There were 16 instances of angular stomatitis in 6 or these subjects. Thirteen of these lesions appeared before supplementation with riboflavin, 8 disappeared before supplementation. The average duration of the fissures which disappeared spontaneously was 4 weeks, while those that did not disappear until after supplementation (2 mg per day) persisted for an average of 7 weeks. In groups "R," "NR" and "TR," comprising 23 subjects (i.e., those supplemented with riboflavin) 7 fissures were seen in 4 subjects. These were considerably less severe than the lesions seen in the subjects of the "U" group and lasted an average of three and one-half weeks.

The other lesion noted with frequency in the subjects of the "U" group was scrotal dermatitis. Erythema and scaling of the scrotum, sparing the medium raphe, were observed in 5 of the 8 subjects. The dermatitis lasted from one to 6 weeks, did not regress spontaneously, but did disappear within from one to 5 weeks following supplementation with riboflavin. Table 2 lists the subjects of the "U" group with the relative

degree of involvement noted before riboflavin (2 mg per day) was added to their regimen. Analyses of riboflavin in the urine which produced data which compared well with those previously reported (Horwitt et al., '49a) will be discussed in a subsequent publication. It is significant that although the present diet differed from that in the previous study in that vitamin B_{12} was provided as a supplement and niacin was quite low, there were no noticeable differences in either the development or the repair of ariboflavinosis.

TABLE 2

Relative pathology of subjects on riboflavin depleted diet

SUBJECT	ORAL CHANGES (Angular stomatitis, glossitis, mucosal erythema)	SCROTAL DERMATITIS	CONJUNCTIVITIS AND SEBORRHEA
U1 (100) [1]	+	+	0
U2 (100)	0	0	0
U3 (90)	+++	++	0
U4 (100)	±	0	0
U5 (100)	++++	++++	++++
U6 (100)	+++	+++	+++
U7 (120)	0	0	0
U8 (100)	+++	++	0

[1] Figures within parentheses indicate the percentage of food allotted.

A patient with the most severe scrotal dermatitis and penile involvement requires special comment. At the time supplementation was begun he showed unilateral angular stomatitis, diffuse erythema involving the tongue and buccal mucosa, retroaural seborrhea, and moderately severe scrotal dermatitis. During the first week after the daily supplementation with 2 mg of riboflavin there suddenly appeared severe blepharitis and conjunctivitis with photophobia, seborrhea and cracking of the skin in the nasolabial folds, marked bilateral angular stomatitis and cheilosis, and the severe penile and scrotal dermatitis described above. These lesions gradually cleared during the next 4 weeks leaving no apparent residue. Another patient with very marked scrotal dermatitis also had seborrhea and conjunctivitis, but to a less severe degree.

Clinical changes not associated with riboflavin deficiency

Physical changes other than those attributed to riboflavin deficiency were not peculiar to any of the groups. No cutaneous lesions resembling those typical of pellagra were seen. Changes in skin sensitivity to single ultra-violet light exposure were not observed. The fore-arms of several of the subjects were exposed to infra-red light and although small third-degree burns were inadvertently produced in three subjects, no resultant adjacent dermatitis occurred.

Nine subjects showed slight to moderate degrees of redness of the anterior portion of the tip of the tongue, with very slight or no papillary atrophy. These included 4 in the "R" group, three in the "NR," one in the "TR" and one member of the "HD" group.

There was little significant variation in blood pressure, heart size, or electrocardiographic tracings. No significant changes were noted in serum cholesterol, N.P.N., gastric hydrochloric acid, urinary sediment or the hematopoietic system. Results on changes in blood lactic and pyruvic acid will be reported elsewhere.

Urinary excretion of nitrogen

The analyses of 596 daily samples of the three basal diets used indicated that they averaged 6.53, 6.39 and 6.58 gm of nitrogen per day, respectively, at the 100% feeding level (table 1). Five of the subjects, U7, R6, TR4, TR6 and TR7, received 120% of this basic level, two, R7 and R8, received 110% and TR8 was allotted 90% of the basal level eaten by the 23 remaining subjects on the controlled dietary intake. At the start of the experiment, the average daily nitrogen excretion for all groups was between 10 and 12 gm of nitrogen (fig. 1). The subjects are not divided into experimental groups in this figure inasmuch as variations in riboflavin, niacin or tryptophan, at the levels studied, had no apparent effect on the amounts of nitrogen excreted. The urinary nitrogen did

not reach minimum levels until after the experimental diet had been fed for more than three months. Thus, after two weeks on the experimental diet, the average excretion of nitrogen in the urine was 6.18 gm for the 100% group, which ate

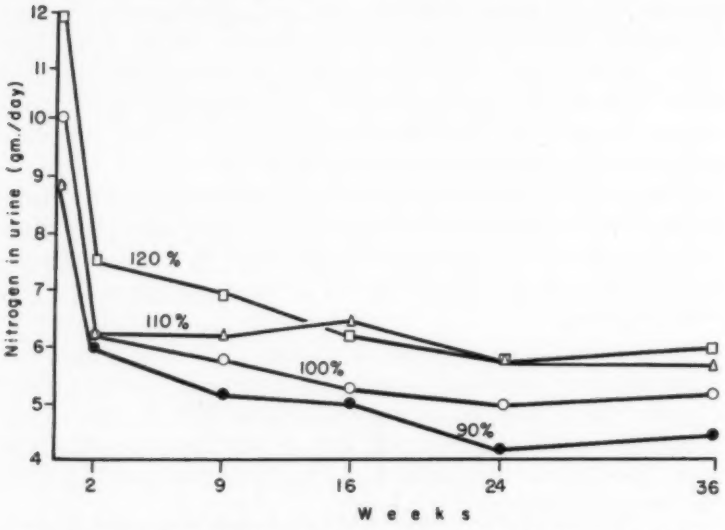

Fig. 1 Average urinary nitrogen excretions at 4 levels of nitrogen intake during first 36 weeks of controlled feeding; the 90, 100, 110 and 120% diets provided average of 5.85, 6.50, 7.15 and 7.80 gm nitrogen per day, respectively.

6.5 gm of nitrogen per day, and 7.54 gm for the 120% group which ate 7.8 gm of nitrogen per day. (The 110 and 90% groups gave parallel data but they represented only two subjects on each intake.) If one assumes that about 1 gm of nitrogen [8] was excreted in the feces, daily, then all these sub-

[8] Such an estimation of fecal nitrogen which is unacceptable in short-term nitrogen balance studies might be considered tolerable under the long-term plan of this experiment. The extra effort and expense that would have been involved in the collection of feces from 40 subjects would have made it impossible to conduct other phases of this project that appeared to show greater promise of producing significant data. Maximum variations in fecal nitrogen that might be expected in men repeating the same diet for many months do not appear to be large enough to have an important effect on the trends observed.

jects were in negative balance on their respective diets at two weeks. This "negative nitrogen balance" continued for at least another 5 weeks with gradual improvement at a rate equivalent to about 0.01 gm of nitrogen per day. Note that this small change per day is difficult to measure and that a short-term metabolic study would have given results indicating that a steady state had been reached, when in fact, this slight change, so insignificant in comparing two successive daily analyses, could continue for weeks, to show a sizeable difference for the longer period. At 109 days, the 21 subjects who received 6.5 gm nitrogen per day (100% diet) had an average urinary excretion of 5.20 mg which might be considered a plateau level. Four subjects who were continued on this basal diet for a much longer time had average daily urinary nitrogen excretions of 5.19, 5.08 and 5.07 gm at 319, 410 and 525 days, respectively (not shown in fig. 1).

In interpreting these data, one should note that body weight changes were relatively small during the entire experiment, that the activity of the subjects did not change from pre-experimental patterns and that the data are uncomplicated by effects of bed-rest (Dietrick et al., '48). Thus the average subject on controlled diets weighed 63 kg at the start of the experiment and 62 kg 250 days later. Tests were made for the presence of edema but increases in body water were not proved. For the following 12 months the picture was complicated, in many of the subjects, by the use of amino acid, choline and protein supplements which were supplied in an effort to determine the factor (or factors) responsible for the liver dysfunctions observed. Table 3 lists the nitrogen excretion of 6 individuals on the 100% diet who were given 10 gm of lactalbumin midway in the experiment. In two analyses made during the ad libitum feeding preliminary period at 81 and 13 days before the start of the experimental diet, a definite relationship was established between these subjects which seems to have been maintained during the later experimental and supplementation periods. One must assume that some of the later differences were due to the individual

TABLE 3

Urinary nitrogen, in grams per day, excreted by those subjects on 100% dietary intake who were supplemented with 10 gm of lactalbumin before being given 30 gm of lactalbumin

WEEKS	PRELIMINARY PERIOD		BASAL DIETS [1]					BASAL DIETS PLUS 10 GM LACTALBUMIN [2] STARTED AT 38TH WEEK		BASAL DIETS PLUS 30 GM LACTALBUMIN [2] STARTED AT 61ST WEEK	
	−12	−2	2	9	16	24	36	46	59	75	85
Subject	*gm*	*gm*	*gm*	*gm*	*gm*	*gm*	*gm*	*gm*	*gm*	*gm*	*gm*
U 4	9.3	10.0	6.2	5.5	5.7	5.3	5.0	6.0	6.5	10.4	7.7
R 2	7.5	8.1	5.3	5.8	5.4	5.6	5.5	5.8	6.6	9.0	7.8
R 5	4.6	5.2	4.6	4.6	3.7	3.6	3.6	4.5	5.4	6.2	6.8
NR 3	8.8	8.3	5.7	5.2	4.9	4.8	4.2	4.6	5.1	7.8	8.3
TR 3	12.9	11.8	5.9	5.8	5.0	4.8	4.5	5.0	. . [4]		
TR 6	10.0	12.4	6.9	6.3	5.7	5.6	5.7	6.5	6.3	9.4	9.0
Average	8.9	9.3	5.8	5.5	5.1	5.0	4.8	5.4	6.0	8.6	7.9

[1] Basal diet provided an average of 6.5 gm of nitrogen.
[2] The 10 gm of lactalbumin provided 1.26 gm of nitrogen.
[3] The daily supplement of 30 gm of lactalbumin was difficult to ingest and these data are probably low.
[4] Subject TR 3 transferred to hospital diet in 52nd week.

variations in fecal nitrogen, but one cannot be certain without complete knowledge of the nitrogen-intake pattern which each subject had maintained during previous years. The length of time it may take to establish such a pattern is not generally appreciated. In the experience of the Elgin investigators, one does not achieve a steady state of urinary nitrogen excretion until at least three months after a major decrease has been made in the level of protein intake; this time factor being dependent on previous levels of protein intake. This fact is not too well appreciated by some investigators who employ short-term nitrogen balance techniques where small changes in nitrogen excretion on successive days are difficult to evaluate. The data in table 3 show that it took approximately 24 weeks to achieve relatively stable urinary nitrogen excretion after the start of the regimen which provided approximately 6.5 gm of nitrogen. Subject R5, who had the lowest excretion during the preliminary period, excreted the least nitrogen at later intervals. In the 46th week, which was 7 weeks after the supplementation with 10 gm of lactalbumin had begun, the nitrogen excretions were considerably lower than on the 19th week of such supplementation (59th week) indicating that even with this relatively small supplement more than 7 weeks were required to achieve a steady state of nitrogen excretion.

Urinary excretion of N^1-methylnicotinamide before lysine, methionine or lactalbumin supplementation

Before the subjects started eating the experimental diet, the group averages for urinary excretion of N^1-Me for subjects on 100% intake fell within the limits of 3.64 mg (R-100) and 4.80 mg (U-100) per day. Figure 2 shows the variations in the averages during the first 36 weeks. Data for later intervals are included in table 4. After 9 weeks on the experimental diet, the excretions of the U-100, R-100 and TR-100 groups had dropped to average values of 1.46, 0.96 and 2.42 mg, respectively, while the NR-100 group (which was supple-

TABLE 4

Urinary excretion of N¹-methylnicotinamide (mg/day)

(U, R, NR, TR and HD refer to original classification of subject as being in the unsupplemented, riboflavin, niacin plus riboflavin, tryptophan plus riboflavin or hospital diet groups, respectively. Data are subgrouped according to percentage of food allotted and similarity of supplementation in 46th week.)

SUBJECT		PRELIMINARY PERIOD	TIME IN WEEKS									
No.	Body wt.		2	9	16	24	36	46 [1]	59	75	84	95 [2]
	kg	*mg/day*	*mg/day*	*mg/day*	*mg/day*	*mg/day*	*mg/day*	*mg/day*	*mg/day*	*mg/day*	*mg/day*	*mg/day*
U2-100	61	4.04	2.49	1.53	2.00	1.37	1.07	0.69 [3]	(0.49) [4]	4.76 [5]	5.57	8.01
U5-100	53	3.76	4.26	0.96	1.20	0.85	1.00	0.64 [3]	1.05 [4]	0.66 [6]	0.69	4.28
U6-100	55	3.66	1.69	1.19	1.29	1.42	0.56	0.49 [7]	0.96 [8]	1.12 [6]	0.91	3.72
U-8-100	59	6.30	5.90	1.56	1.53	0.77	0.86	0.98 [3]	1.29 [4]	... [9]	...	...
U3-90	63	5.39	1.63	2.06	1.10	1.96	1.28	6.06 [10]	3.25 [11]	6.24 [12]	6.50	9.14
U1-100	69	7.63	2.47	2.37	2.10	1.42	1.49	1.81 [13]	2.61	3.14 [14]	3.33	7.03
U4-100	63	3.42	2.23	1.16	1.10	1.25	0.60	2.04 [13]	2.33	5.01 [5]	2.96	3.00
U7-120	65	5.49	3.61	1.68	2.03	2.67	1.63	1.61 [13]	2.13	3.87 [15]	4.79	4.84
R1-100	61	5.41	2.54	1.18	1.33	0.96	0.47	0.38 [7]	0.63 [8]	... [9]	...	...
R3-100	59	3.15	1.84	1.28	1.39	1.11	1.40	0.87 [3]	1.23 [4]	1.38 [6]	1.34	8.43
R4-100	58	3.44	2.09	0.59	1.07	0.88	0.87	0.82 [7]	0.59 [8]	0.98 [6]	0.81	3.86
R6-120	71	5.69	3.22	2.54	1.75	1.76	1.17	0.77 [3]	0.75 [4]	1.87 [14]	2.11	3.08
R2-100	64	2.58	1.55	0.72	0.61	(0.26) [16]	(1.04) [16]	0.61 [13]	0.72	3.00 [14]	1.93	8.15
R5-100	65	...	2.59	1.04	0.96	0.80	0.92	(0.55) [13]	1.09	1.47 [14]	1.91	2.40
R8--110	55	5.16	2.37	1.60	1.21	0.75	1.05	1.92 [13]	1.42	1.45 [6]	1.20	5.18
NR1-100	73	4.69	6.37	5.89	5.36	5.27	6.12	3.22 [7]	2.79 [8]	5.47 [14]	5.53	6.02
NR2-100	62	4.87	6.91	4.99	6.90	4.25	4.00	4.03 [3]	4.27 [11]	9.65	7.03	8.45
NR5-100	61	3.87	3.36	4.11	3.22	2.81	3.38	2.68 [7]	2.55 [8]	2.36 [6]	3.57	5.93
NR4-120	69	5.05	6.78	5.47	5.15	3.32	4.04	4.72 [3]	5.47 [4]	6.98 [15]	5.98	5.62
NR7-120	77	...	4.78	3.08	4.83	4.76	3.16	3.57 [3]	6.23 [4]	5.76 [14]	4.44	5.06
NR3-100	58	3.44	6.24	6.56	5.09	4.55	3.52	5.43 [13]	5.34	7.23 [14]	6.47	8.48
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
TR4-100	61	3.64	2.03	1.88	1.11	1.71	1.86	1.12 [3]	1.40 [4]	1.55 [15]	2.41	4.40
TR5-100	76	5.64	1.58	2.47	1.80	2.23	1.46	1.17 [7]	1.48 [8]	6.15 [17]	4.14	5.39
TR7-100	76	4.90	2.12	1.83	2.16	2.43	1.84	1.03 [3]	1.74 [4]	3.65 [14]	3.41	6.87

NR3-100	58	3.44	6.24	6.56	5.09	4.55	3.52	5.43 [13]	5.34	7.23 [14]	6.47	8.48
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
TR4-100	61	3.64	2.03	1.88	1.11	1.71	1.80	1.12 [9]	1.40 [4]	1.55 [15]	2.41	4.40
TR5-100	76	5.64	1.58	2.47	1.80	2.23	1.46	1.17 [7]	1.48 [8]	6.15 [17]	4.14	5.39
TR7-100	76	4.90	2.12	1.83	2.16	2.43	1.84	1.03 [8]	1.74 [4]	3.65 [14]	3.41	6.87
TR8-90	57	5.39	2.16	2.00	1.68	2.19	1.32	1.31 [7]	0.96 [8]	3.33 [14]	2.61	7.44
TR2-110	68	2.56	3.03	3.29	1.82	1.86	1.19	... [9]	...	...	...	...
TR3-100	65	4.16	4.03	3.43	2.73	1.02	1.76	1.83 [13]	5.54 [11]	8.33	9.34	7.13
TR6-100	62	4.87	2.58	3.09	1.51	2.12	1.46	1.69 [13]	1.85	4.27 [14]	2.50	6.84
HD1	70	3.16	...	...	...	...	...	2.59 [13]	3.37	3.36	3.45	4.97
HD2	54	3.65	...	4.57	4.10	3.85	6.00	3.99 [13]	3.79	6.20	4.02	3.57
HD3	63	1.50	...	5.48	5.83	4.39	3.87	5.39 [13]	5.17	3.68	3.42	3.41
HD4	70	...	...	...	...	...	...	5.03 [13]	4.52	6.32	6.03	10.42
HD5	65	6.02	...	8.96	8.57	8.84	6.56	4.17 [13]	4.37	8.10	7.55	8.86
HD6	57	7.33	...	6.74	8.88	7.28	10.12	5.19 [13]	4.36	6.55	4.95	7.34
HD7	62	5.92	...	...	...	...	...	7.41 [13]	3.64	5.62	...	...
HD8	53	3.64	...	...	...	...	...	2.37 [13]	2.61	5.21	3.61	4.25
HD9	72	3.79	...	...	...	...	...	6.47 [13]	6.22	4.83	4.04	5.69

[1] In 39th week U-group received 2 mg additional riboflavin (same as R-group).

[2] All subjects returned to ad libitum feeding with increased meat ration in 88th week.

[3] Supplemented with 1 gm of L-lysine per day in 39th week and continued until 52nd week (subgroup II).

[4] Zein in diet replaced by lactalbumin supplying equal amount of nitrogen from 52nd week to 61st week (subgroup II).

[5] Placed on hospital diet in 69th week.

[6] Folic acid, calcium pantothenate, pyridoxine, and vitamin B_{12} removed from diet in 61st week.

[7] Supplemented with 330 mg of DL-methionine in 39th week and continued until 52nd week (subgroup III).

[8] Methionine supplementation ceased in 52nd week and supplemented with 1.3 gm of choline dihydrogen citrate daily from 52nd week to 61st week.

[9] Removed from project.

[10] Placed on high protein diet following surgery in 42nd week.

[11] Placed on hospital diet in 52nd week.

[13] In General Hospital 69th to 88th week.

[14] Supplemented with 10 gm of lactalbumin in 39th week (subgroup I).

[15] Supplemented with 30 gm of lactalbumin in 61st week.

[16] Supplemented with 30 gm of lactalbumin in 65th week.

[16] Results in parentheses considered unreliable.

[17] Placed on hospital diet in 65th week (high protein).

[18] Supplementation of HD group with Vi-Penta tablets ceased in 38th week.

mented with 10 mg of niacinamide) increased to 5.39 mg per day. At the same time, the HD group which received the hospital diet plus a supplement of 10 mg of niacinamide, three times a week, reached a plateau level of 6.44 mg.

The R-100 group was close to its minimum average excretion level at the 9th week with only minor fluctuations thereafter. The U-100 group average took somewhat longer to come

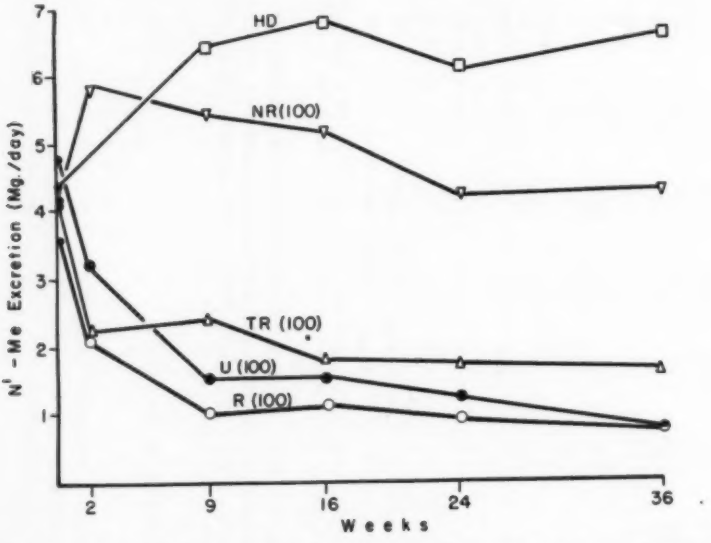

Fig. 2 Average daily excretion of N^1-methylnicotinamide of the experimental groups.

down to a similar low level of about 1.0 mg N^1-Me per day but this time difference is probably related to the higher initial reserve shown by the U-100 subjects in the preliminary period. At two weeks the average values for the TR-100 (2.30 mg), U-100 (3.17 mg) and R-100 (2.12 mg) groups were still a function of the pre-experimental levels. The data from the 9th week gave definite confirmation of the conversion of tryptophan to N^1-Me even though the L-tryptophan supplement was only 50 mg per day at that time. L-Tryptophan supple-

mentation of the TR-100 group was raised from 50 to 100 mg during the 10th week. The importance of initial reserves of niacin cannot be overemphasized in evaluating any depletion experiment in man that has a duration of less than several months.

The initial sharp rise in N^1-Me excretion by the NR-100 group was not maintained. This may be due to the fact that when niacinamide was first given to these individuals, they had good niacin reserves which were gradually diminished as they continued on the basal diet plus the niacin supplement. These data may indicate that this basal diet plus 10 mg of niacin represented no more total niacin than that which had been their intake on the hospital diet. Thus, after 24 weeks, the NR-100 group was not excreting any more N^1-Me than at the start of the experiment despite the fact that they were receiving the niacin supplement. There is the possibility that the decrease in N^1-Me excretion during the first few months was partially ameliorated by tissue products due to the slight negative nitrogen balance which existed at that time. Reference to figure 1 will show that urinary nitrogen excretion was not stabilized until the 24th week in the groups on 100% intake.

The high results in the HD group (HD2, HD3, HD5 and HD6) were due to the administration of a commercial vitamin capsule [9] three times a week in conjunction with ingestion of about 75 gm of protein, daily. When the vitamin supplementation of this group was discontinued at 38 weeks, the N^1-Me excretions dropped back to their pre-experimental levels of about 4.3 mg per day.

Evaluation of the data in table 4 must take into consideration the fact that, in each experimental group, there were some subjects who received more or less than 100% of the basal diet. The difference in tryptophan and niacin ingested by a subject on 100% intake (265 mg of tryptophan and 5.8 mg of niacin) and one on 120% intake was approximately 53 mg of tryptophan and 1.2 mg of niacin, respectively. Thus, a subject

[9] See footnote 6.

in an unsupplemented group (U or R group) who received 120% of the basal diet was being allotted 318 mg of tryptophan, which is not much less than 338 mg of tryptophan which the subject on 90% intake was receiving in the TR group (basal diet plus 100 mg of tryptophan).

Table 4 shows that several of the subjects had low excretions of N^1-Me which approached the upper limits of those reported by Goldsmith et al. ('52), levels which were associated with clinical pellagra on a corn diet. In the latter study, excretions of 0.5 and 0.6 mg of N^1-Me were obtained in 50 to 60 days. In the present study, only one subject (R2-100) excreted less than 0.96 mg at 16 weeks and although, between 46 and 59 weeks, 6 subjects (U2-100, U5-100, U6-100, R1-100, R2-100 and R5-100) recorded excretions of 0.6 mg or less, clinical evidence of pellagra was not apparent. These results are not inconsistent with similar observations on wheat diets which were being conducted at Tulane University (Goldsmith et al., '52) at about the same time. In the latter study, the depletion period was 95 days at which time some of the subjects in the Elgin study had also reached their minimal levels of N^1-Me excretion.

Effects of lysine supplementation on N^1-methylnicotinamide excretion

The apparent changes in N^1-Me excretion observed (table 4, footnote 10) when 1 gm of L-lysine per day was given (39th week) to subjects U2-100, U5-100, R3-100 and R6-120 deserve special comment. These subjects had a decrease in N^1-Me excretion, presumably due to the amino acid imbalance associated with lysine supplementation (Koeppe and Henderson, '55). These suggestions of decreases were in contrast to larger excretions obtained at the same time, in 6 subjects in the same group who were supplemented with 10 gm of lactalbumin per day, although two of these subjects (R2-100 and R5-100) needed more time to show the ameliorative effect of the lactalbumin. The two subjects in the TR group (TR4-100 and TR7-100) who received 1 gm of L-lysine per day

had marked decreases in their N^1-Me excretion, but no effects of lysine supplementation on the NR group were apparent.

Effects of methionine and choline supplementation on N^1-methylnicotinamide excretion

The addition of 330 mg of DL-methionine per day to 8 subjects during the 39th week had effects on the N^1-Me excretion which were qualitatively similar to those obtained with L-lysine (table 4, footnote 11). Although each subject given methionine subsequently had a lower excretion of N^1-Me, the changes obtained were insignificant in the U and R group as these subjects were already excreting very little N^1-Me during the interval which preceded methionine supplementation. One of the two subjects in the NR group (NR1-100 and NR5-100) who received methionine showed a decrease in N^1-Me excretion.

All of the subjects who had been receiving methionine were changed to supplementation with choline dihydrogen citrate (1.30 gm per day) in the 52nd week. (This was done to decrease the high retention of bromsulfalein and was not directly related to the studies of N^1-Me at that time.) While no definitive changes in N^1-Me excretion were observed during the period of choline supplementation, the pattern obtained with the previous supplementation with methionine was continued.

Effects of lactalbumin supplementation on N^1-methylnicotinamide excretion

As mentioned above, the addition of 10 gm of lactalbumin during the 38th week (table 4, footnote 7) produced increases in the excretion of N^1-Me. In comparing the effects of supplementation with L-tryptophan or lactalbumin one can select the subjects in the TR group who were not supplemented with lactalbumin and compare them with those in the U and R groups who received lactalbumin. The tryptophan content of

the 10 gm of lactalbumin used [10] was approximately 200 mg. As the amount of tryptophan in 10 gm of lactalbumin is about twice the 100 mg added daily to the basal diet given to the subjects in the TR group, the increased efficiency of the lactalbumin supplementation on N^1-Me can be calculated as a function of the additional tryptophan so provided. To estimate the comparative value of tryptophan from lactalbumin and of the 10 mg of niacin given to the NR subjects, one can compare the pre-lactalbumin supplementation data from the 36th week with data obtained during the 59th week. The average increase in the excretion of N^1-Me due to the addition of 10 gm of lactalbumin in the 39th week to 6 subjects who appear to have given reliable data (U1-100, U4-100, U7-120, R8-110, NR3-100 and TR6-100) was approximately 1 mg per day. The difference between the amounts excreted by the NR-100 and U and R-100 groups was approximately 3 mg per day (see fig. 2). On this basis one might regard 1 mg of niacin as being equivalent to about 3 gm of lactalbumin or to about 60 mg of tryptophan provided in the form of lactalbumin. While this calculated result cannot be proved statistically significant from the data presented, it can serve as a reference point for estimating the niacin equivalence of tryptophan-containing compounds; some additional justification for this relationship is presented below in the section in which N^1-Me excretion produced by ingestion of niacin or tryptophan is discussed. When lactalbumin replaced zein in the basal diet (52nd to 61st week, table 4, footnote 12), subjects U5-100, U8-100 and R3-100 showed increases in N^1-Me excretion of 0.64 to 1.05, 0.98 to 1.29 and 0.87 to 1.23 mg, respectively, after 7 weeks. Since this tryptophan addition of approximately 140 mg per day followed the cessation of lysine supplementation, these data are difficult to evaluate.

Urinary excretion of quinolinic acid, niacin and tryptophan

The excretion data for quinolinic acid indicate that this analysis, though of theoretical importance, does not produce

[10] Labco Brand, purchased from The Borden Company (nitrogen content 12.6%).

as good correlations with tryptophan ingestion as does N^1-Me. Table 5 records all the quinolinic acid data obtained in this project. There seem to be few significant changes in the average quinolinic acid excretion of each group after the first few weeks of the experiment. Quinolinic acid excretion is not increased much by supplementation with either nicotinic acid or 10 gm of lactalbumin, but it is increased markedly by feeding 1 gm doses of tryptophan (Goldsmith et al., '52). Quinolinic acid or the method of assay used is not a good index of niacin deficiency. In comparing the data reported here with those from other laboratories, it should be noted that some of the older reports give much lower figures for quinolinic acid in urine inasmuch as the assays were performed before it was known that autoclaving with 1 N sulfuric acid was not as efficient in converting quinolinic acid to niacin as autoclaving with glacial acetic acid (Sarett, '51).

There were no significant changes in the levels of niacin excretion in the urine (table 6). These data show that niacin in urine is not directly related to the nutritional state of the subject.

The urinary excretions of tryptophan (table 7) seemed to be about the same for all groups and did not change appreciably during depletion periods. However, despite the tendency for each subject to retain a pattern of tryptophan excretion which, as with the other amino acids, appears to be an individual characteristic (Brewer et al., '47; Harvey and Horwitt, '49) there was an increase in tryptophan excretion when the subjects were placed on a diet high in animal protein (95th week). As the individual differences in tryptophan excretion on a given diet were quite marked, this test has not been useful in the evaluation of niacin-tryptophan requirements. For what it is worth, it may be noted that after 36 weeks of depletion, none of the subjects in the depleted groups had excretions greater than 16 mg of tryptophan per day, whereas, excretions of over 20 mg were not infrequent in the subjects receiving better diets.

TABLE 5

Urinary excretion of quinolinic acid (mg/day)

SUBJECT No.	SUBJECT Body wt.	PRELIMINARY PERIOD	TIME IN WEEKS 2	9	16	24	36	46 [1]	59	75	84	95 [2]
	kg	*mg/day*	*mg/day*	*mg/day*	*mg/day*	*mg/day*	*mg/day*	*mg/day*	*mg/day*	*mg/day*	*mg/day*	*mg/day*
U2-100	61	3.88	4.56	4.33	4.46	2.92	3.60	4.51 [3]	3.29 [4]	3.68 [5]	3.78	3.55
U5-100	53	2.73	4.35	2.35	3.51	1.97	2.45	2.43 [3]	2.50 [4]	2.53 [6]	1.37	3.68
U6-100	55	3.19	3.31	2.23	3.60	3.56	3.01	2.67 [7]	2.62 [3]	2.87 [6]	1.80	2.38
U8-100	59	6.63	4.87	3.42	5.05	3.45	3.57	3.48 [3]	3.05 [4]	... [9]	...	...
U3-90	63	3.77	3.66	1.94	4.04	1.87	2.26	... [10]	... [11]	2.55 [12]	1.68	2.94
U1-100	69	3.87	4.14	2.98	4.53	2.26	3.09	3.72 [13]	4.42	2.10 [14]	3.73	3.39
U4-100	63	3.33	3.63	2.50	4.43	1.96	2.63	3.60 [13]	3.10	3.17 [5]	2.58	3.16
U7-120	65	5.02	5.00	3.99	4.59	4.42	4.47	5.05 [13]	3.84	3.69 [15]	4.14	4.12
R1-100	61	3.29	3.45	1.87	3.42	2.18	3.25	2.51 [7]	2.03 [3]	... [9]	...	7.73
R3-100	59	4.19	4.13	4.19	6.29	3.60	4.26	3.43 [3]	3.69 [4]	3.36 [6]	3.00	2.45
R4-100	58	3.15	3.76	3.00	4.12	2.86	3.86	3.48 [7]	1.74 [3]	2.97 [6]	2.48	2.96
R6-120	71	3.55	4.25	4.22	7.12	4.06	3.62	3.86 [3]	2.81 [4]	3.87 [14]	3.62	2.02
R2-100	64	3.07	3.55	3.21	4.66	3.41	4.74	3.54 [13]	2.59	3.11 [14]	3.28	3.25
R5-100	65	1.84	3.16	1.89	3.65	2.63	2.47	2.56 [13]	2.27	3.01 [14]	2.69	1.92
R8-110	55	4.91	4.62	3.42	4.75	2.90	4.34	3.91 [13]	4.16	4.12 [6]	4.06	4.33
NR1-100	73	3.82	4.38	3.30	4.28	2.61	3.59	3.28 [7]	1.12 [3]	4.09 [14]	3.41	3.91
NR2-100	62	6.30	8.61	7.88	7.53	8.61	6.55	6.46 [3]	7.57 [11]	6.72	7.32	6.81
NR5-100	61	3.00	3.92	2.79	4.18	3.91	3.83	3.44 [7]	2.84 [3]	2.51 [6]	2.84	3.98
NR4-120	69	4.70	4.62	3.61	5.12	6.01	4.42	4.08 [3]	4.11 [4]	4.33 [15]	4.88	4.71
NR7-120	77	2.49	4.20	3.26	4.97	4.60	3.53	3.76 [3]	3.05 [4]	2.75 [14]	3.84	5.93
NR3-100	58	4.63	6.01	5.15	5.67	3.83	5.10	5.35 [13]	3.77	4.61 [14]	5.12	6.02
NR6-120	63	4.32	5.41	3.84	5.04	4.52	4.69	5.51 [13]	4.22	3.49 [14]	4.77	3.88
TR1-100	58	3.68	5.19	4.96	3.42	4.89	3.48	4.12 [7]	2.62 [3]	1.26 [14]	3.66	4.02
TR4-100	61	5.53	5.88	4.64	3.91	5.54	5.06	5.23 [3]	3.71 [4]	4.84 [14]	4.99	4.41
TR5-100	76	4.69	4.40	3.95	4.22	3.13	4.51	4.22 [7]	3.26 [3]	3.68 [17]	8.81	4.44
TR7-100	76	5.28	4.43	5.36	3.95	4.60	4.70	3.76 [3]	3.65 [4]	3.93 [14]	4.89	5.58
TR8-90	57	4.02	4.60	3.73	3.91	3.73	3.71	3.70 [7]	2.39 [3]	3.97 [14]	3.75	3.91
TR2-110	68	3.90	4.86	5.09	4.87	3.36	3.43	... [9]	...	...	...	...
TR3-100	65	3.92	3.79	3.02	3.34	3.79	4.19	3.65 [13]	3.08 [11]	2.62	3.57	4.15
TR6-100	62	4.45	4.03	3.63	3.57	4.20	3.35	4.64 [13]	3.65	3.75 [14]	2.84	4.15
HD1	70	4.18	...	...	...	...	...	3.87 [13]	4.48	4.98	5.46	4.09
HD2	54	3.68	...	3.95	3.46	4.83	4.41	4.17 [13]	3.02	4.87	3.52	3.57
HD3	63	3.67	...	3.91	3.43	3.83	3.00	3.71 [13]	6.02	3.85	2.86	4.23
HD4	70	...	...	...	...	...	...	5.47 [13]	5.42	7.47	5.12	8.57
HD5	65	3.55	...	4.58	4.40	4.34	3.72	4.39 [13]	2.85	4.62	3.86	3.97
HD6	57	2.88	...	3.99	4.67	4.10	3.73	3.55 [13]	3.21	3.27	3.50	3.66
HD7	62	4.22	...	...	...	...	...	1.87 [13]	3.28	2.69	...	...
HD8	53	3.74	...	...	...	...	...	3.80 [13]	3.32	7.99	8.50	12.06
HD9	72	4.75	...	...	...	...	...	10.34 [13]	4.97	4.43	3.39	7.57

Urinary excretion of free niacin

HD8	53	3.74	...	...	...	...	...	3.80 [13]	3.32	7.99	8.50	12.06
HD9	72	4.75	...	...	...	...	...	10.34 [13]	4.97	4.43	3.39	7.57

Urinary excretion of free niacin

SUBJECT		PRELIMINARY PERIOD	TIME IN WEEKS									
No.	Body wt.		2	9	16	24	36	46 [1]	59	75	84	95 [2]
	kg	*mg/day*	*mg/day*	*mg/day*	*mg/day*	*mg/day*	*mg/day*	*mg/day*	*mg/day*	*mg/day*	*mg/day*	*mg/day*
U2-100	61	0.80	0.54	0.74	0.55	0.62	0.44	0.68 [3]	0.79 [4]	0.84 [5]	0.79	0.86
U5-100	53	0.56	0.45	0.53	0.45	0.34	0.37	0.49 [3]	0.98 [4]	0.39 [5]	0.61	0.60
U6-100	55	0.65	0.47	0.53	0.45	0.55	0.33	0.45 [7]	0.57 [8]	0.56 [6]	0.61	0.68
U8-100	59	0.87	0.52	0.58	0.47	0.55	0.30	0.62 [3]	0.71 [4]	... [9]	...	...
U3-90	63	0.72	0.61	0.87	0.63	0.57	0.36	1.02 [10]	0.88 [11]	0.87 [12]	1.07	0.95
U1-100	69	0.77	0.58	0.67	0.64	0.64	0.49	0.61 [13]	0.96	0.70 [14]	0.60	0.84
U4-100	63	0.61	0.41	0.52	0.46	0.54	0.33	0.53 [13]	0.61	0.69 [5]	0.61	0.56
U7-120	65	0.84	0.70	0.84	0.69	0.84	0.59	0.76 [13]	0.98	0.86 [15]	1.41	1.00
R1-100	61	0.78	0.58	0.52	0.45	0.54	0.35	0.46 [7]	0.54 [3]	... [9]	...	0.69
R3-100	59	0.64	0.66	0.79	0.59	0.65	0.47	0.50 [3]	0.87 [4]	0.56 [6]	0.24	0.65
R4-100	58	0.62	0.45	0.49	0.49	0.57	0.39	0.47 [7]	0.64 [3]	0.47 [6]	0.62	0.70
R6-120	71	0.89	0.75	0.68	0.68	0.82	0.50	0.60 [3]	0.85 [4]	0.70 [14]	0.88	0.62
R2-100	64	0.53	0.54	0.51	0.60	0.82	0.48	0.52 [13]	0.94	0.54 [14]	0.93	0.79
R5-100	65	0.44	0.72	0.61	0.58	0.56	0.42	0.68 [13]	0.94	0.53 [14]	0.91	0.75
R8-110	55	0.60	0.56	0.54	0.64	0.62	0.43	0.52 [13]	0.84	0.67 [6]	0.80	0.77
NR1-100	73	0.72	0.71	0.61	0.69	0.74	0.69	0.74 [7]	0.80 [3]	0.79 [14]	0.90	0.90
NR2-100	62	0.70	0.76	0.60	0.63	0.70	0.61	0.69 [3]	0.86 [11]	0.58	0.95	0.77
NR5-100	61	0.54	0.58	0.61	0.71	0.72	0.63	1.01 [7]	0.69 [3]	0.61 [6]	0.83	0.90
NR4-120	69	0.88	0.80	0.66	0.76	0.83	0.56	0.76 [3]	0.73 [4]	0.52 [15]	1.18	0.97
NR7-120	77	1.06	0.77	0.65	0.71	1.06	0.57	1.06 [3]	1.30 [4]	0.84 [14]	1.16	0.87
NR3-100	58	0.72	0.66	0.62	0.61	0.82	0.54	0.67 [13]	0.79	0.67 [14]	0.97	0.91
NR6-120	63	0.80	0.63	0.61	0.72	0.81	0.72	0.94 [13]	1.21	0.71 [14]	0.97	0.92
TR1-100	58	0.56	0.48	0.64	0.52	0.62	0.42	0.53 [7]	0.63 [3]	0.60 [14]	0.89	0.79
TR4-100	61	0.66	0.54	0.49	0.52	0.61	0.48	0.54 [3]	0.69 [4]	0.44 [14]	0.74	0.74
TR5-100	76	0.66	0.50	0.49	0.50	0.76	0.50	0.52 [7]	0.82 [3]	0.41 [17]	0.78	0.74
TR7-100	76	0.72	0.43	0.59	0.51	0.58	0.55	0.50 [3]	0.70 [4]	0.44 [14]	0.78	0.77
TR8-90	57	0.70	0.55	0.51	0.53	0.58	0.51	0.54 [7]	0.65 [3]	0.46 [14]	0.66	0.78
TR2-110	68	0.60	0.49	0.68	0.72	0.76	0.59	... [9]	...	...	...	...
TR3-100	65	0.74	0.60	0.60	0.62	0.59	0.56	0.75 [13]	0.80 [11]	0.74	0.80	0.81
TR6-100	62	0.62	0.60	0.57	0.53	0.64	0.56	0.62 [13]	0.80	0.64 [14]	0.88	0.85
HD1	70	0.64	...	...	...	...	...	0.59 [13]	0.80	0.59	0.70	0.66
HD2	54	0.52	...	0.41	0.58	0.55	0.69	0.62 [13]	0.92	0.63	0.70	0.67
HD3	63	0.64	...	0.50	0.74	0.61	0.57	0.72 [13]	0.83	0.57	0.83	0.64
HD4	70	...	...	...	...	...	...	0.85 [13]	1.12	0.75	1.26	0.92
HD5	65	0.82	...	0.54	0.70	0.74	0.75	0.61 [13]	0.81	0.79	1.26	1.11
HD6	57	0.70	...	0.53	0.63	0.52	0.69	0.58 [13]	0.82	0.77	0.69	0.88
HD7	62	0.57	...	...	...	...	...	0.91 [13]	0.80	0.58	...	...
HD8	53	0.65	...	...	...	...	...	0.44 [13]	0.72	0.54	0.79	0.74
HD9	72	0.72	...	...	...	...	...	0.77 [13]	0.83	0.51	1.78	0.85

See table 4 for description of footnotes.

TABLE 7

Urinary excretion of L-*tryptophan*

SUBJECT No.	SUBJECT Body wt.	PRELIMINARY PERIOD	TIME IN WEEKS 2	9	16	24	36	46 [1]	59	75	84	95 [2]
	kg	*mg/day*	*mg/day*	*mg/day*	*mg/day*	*mg/day*	*mg/day*	*mg/day*	*mg/day*	*mg/day*	*mg/day*	*mg/day*
U2-100	61	9.6	8.9	11.9	10.8	9.1	9.1	10.6 [3]	6.0 [4]	26.7 [5]	16.0	12.6
U5-100	53	7.0	6.4	11.6	7.8	6.7	8.4	6.8 [3]	.. [4]	9.4 [6]	10.5	11.7
U6-100	55	10.0	12.0	13.2	12.6	11.7	12.2	12.0 [7]	7.4 [3]	15.7 [6]	14.3	17.2
U8-100	59	7.4	14.2	10.0	8.4	9.4	8.7	8.0 [3]	4.8 [4]	... [9]	...	...
U3-90	63	10.3	10.3	11.7	10.2	10.3	10.4	12.6 [10]	... [11]	23.6 [12]	17.8	16.4
U1-100	69	23.9	18.8	14.1	13.0	12.3	14.5	16.8 [13]	18.5	20.0 [14]	12.4	15.9
U4-100	63	11.9	14.7	16.6	14.2	13.7	13.0	16.4 [13]	17.7	18.6 [5]	11.5	13.3
U7-120	65	13.2	16.6	15.6	13.0	14.9	14.8	17.6 [13]	17.6	17.5 [15]	23.9	21.1
R1-100	61	12.1	18.7	12.9	11.4	12.4	13.4	12.0 [7]	20.0 [3]	... [9]	...	26.0
R3-100	59	9.2	13.0	11.1	8.0	14.4	8.1	7.4 [3]	6.7 [4]	5.9 [6]	8.3	15.5
R4-100	58	12.6	19.5	16.3	13.4	13.2	16.2	13.5 [7]	8.8 [3]	7.2 [6]	14.7	25.5
R6-120	71	7.5	10.8	12.4	9.0	8.3	7.9	8.0 [3]	6.8 [4]	18.3 [16]	10.0	8.6
R2-100	64	6.7	6.7	11.3	8.8	9.4	8.3	8.2 [13]	..	9.5 [14]	14.7	12.9
R5-100	65	5.9	12.3	10.8	6.8	8.8	7.4	9.3 [13]	5.6	8.8 [14]	9.2	14.0
R8-110	55	7.4	8.7	11.8	9.5	8.4	6.1	8.8 [13]	10.2	11.6 [6]	11.9	14.2
NR1-100	73	19.3	22.7	18.8	17.5	16.7	16.8	14.9 [7]	9.9 [3]	8.1 [14]	19.8	25.2
NR2-100	62	8.0	7.8	8.4	6.0	6.9	5.6	4.9 [3]	6.8 [11]	12.9	9.3	27.5
NR5-100	61	15.8	9.9	17.0	10.1	11.6	9.1	9.2 [7]	18.0 [3]	11.2 [6]	9.0	15.9
NR4-120	69	9.3	16.5	14.3	12.7	11.4	11.2	11.3 [3]	5.3 [4]	19.4 [15]	14.2	17.3
NR7-120	77	14.9	13.7	20.8	19.4	24.0	19.2	19.8 [3]	18.4 [4]	21.9 [14]	18.5	24.3
NR3-100	58	15.2	11.7	10.8	8.7	9.7	8.4	7.8 [13]	12.2	11.9 [14]	10.4	13.2
NR6-120	63	12.1	16.6	13.2	10.8	12.8	12.1	15.1 [13]	18.0	12.6 [14]	12.0	18.4
TR1-100	58	7.8	11.8	13.7	7.8	7.7	7.3	7.1 [7]	7.2 [3]	9.9 [14]	7.5	15.2
TR4-100	61	11.8	19.0	17.3	14.4	24.6	17.8	14.8 [3]	6.1 [4]	16.2 [14]	20.0	31.0
TR5-100	76	19.2	20.9	20.2	18.6	23.8	22.2	22.2 [7]	19.4 [3]	16.6 [17]	20.0	28.6
TR7-100	76	12.4	16.3	19.4	14.9	12.9	14.5	11.8 [3]	11.3 [4]	18.4 [14]	15.9	20.4
TR8-90	57	7.6	7.8	14.0	7.5	7.6	7.8	8.9 [7]	8.5 [3]	10.3 [14]	8.2	11.1
TR2-110	68	10.1	9.8	17.0	12.2	10.3	12.8	.. [9]	..	....	..	...
TR3-100	65	12.2	13.4	11.4	9.1	12.3	12.0	11.4 [13]	13.3 [11]	12.5	11.6	14.3
TR6-100	62	7.9	15.1	12.0	8.7	8.3	8.2	9.4 [13]	5.0	10.7 [14]	12.1	13.8
HD1	70	9.8	...	...	...	...	...	17.1 [13]	16.9	18.5	18.6	16.2
HD2	54	8.2	...	30.6	11.6	14.5	18.6	14.5 [18]	...	20.2	12.3	17.8
HD3	63	11.7	...	31.6	35.6	20.2	20.5	23.6 [13]	...	20.6	18.2	26.7
HD4	70	9.2	...	...	...	...	...	11.4 [18]	...	13.6	13.0	14.0
HD5	65	7.1	...	16.6	11.7	9.7	9.8	10.0 [13]	...	10.7	12.2	15.0
HD6	57	10.9	...	18.5	15.0	13.2	13.6	15.6 [13]	...	22.8	20.0	18.1
HD7	62	7.7	...	...	...	...	...	21.3 [13]	...	17.3	...	...
HD8	53	7.2	...	...	...	...	...	10.2 [18]	16.3	14.4	12.0	12.1
HD9	72	10.0	...	...	...	...	...	19.2 [13]	...	17.8	23.4	17.5

Urinary excretion of 6-pyridone of N^1-methylnicotinamide and xanthurenic acid

The determination of 6-pyr may be quite reliable when conducted on urines from individuals receiving large excesses of niacin or tryptophan, and 6-pyr may be a preferred excretion path when large amounts of niacinamide or tryptophan are administered, but the assay of this compound in urines from individuals on low or borderline diets is relatively difficult. Thus, insignificant amounts of 6-pyr were found in urines collected during the pre-experimental period and within a few days after the experimental diet started. Furthermore, the addition of 100 mg of tryptophan a day to the basal diet did not raise the excretion of 6-pyr a measurable amount. Some of the difficulties encountered may be related to the high and variable blank, which is an integral part of the method and which this laboratory could not minimize in the time available. Agreement between duplicate analyses by different workers on different days was poor. This experience, while not inconsistent with personal communications from other laboratories, should not eliminate consideration of the possibility that future work may improve the technique and permit better measurement of small amounts of 6-pyr in urine. It is of interest to note that Goldsmith et al. ('52) found "non-detectable amounts" of 6-pyr in the urine of their subjects after 22 days on their niacin-deficient diets and that when one of their subjects was supplemented with 2 mg niacinamide per day for three months the 6-pyr remained "non-detectable" despite the fact that the N^1-Me excretion did not decrease below 0.9 mg per day on this regimen.

Xanthurenic acid was determined in all urines collected during the first 24 weeks of the depletion period. The amounts in all groups were similar, the U, R, NR, TR and HD groups averaging 12.9, 12.0, 15.5, 12.4 and 15.3 mg per day, respectively. This agrees with previous results (Sarett and Goldsmith, '50).

Effects of withdrawal of folic acid, calcium pantothenate, pyridoxine and vitamin B_{12} from the vitamin supplement

As clinical signs of pellagra had not appeared in any of the subjects by the 62nd week, folic acid, calcium pantothenate, pyridoxine and vitamin B_{12} were removed at this time from the vitamin supplement of 6 of the subjects who were not receiving any lactalbumin. This was done to determine whether these vitamins were preventing the development of a pellagra syndrome. These subjects (U5-100, U6-100, R3-100, R4-100, R8-110 and NR5-100) were chosen for observation of possible effects of prolonged feeding of the basal diet because they had not shown any evidence of liver dysfunction, as determined by bromsulfalein retention test, during the course of the project and therefore had not received any protein supplements. During the following 25 weeks, these subjects showed no significant alterations in their previous clinical or biochemical patterns. It should be noted that two subjects (U6-100 and R4-100) received the basal 100% diet (5.6 mg of niacin and 265 mg% tryptophan) for 87 weeks without any symptoms of pellagra becoming evident. Two other subjects (U5-100 and R3-100) received the basal diet for the same 87-week period except that 7 gm of lactalbumin replaced 7 gm of zein in their diet from the 52nd to 61st week.

Comparison of increases in N^1-methylnicotinamide excretion produced by tryptophan and by niacin

The importance of evaluating tryptophan as a substitute or supplement for niacin should be apparent as any estimation of the niacin equivalence of a given diet must depend upon the amount of tryptophan in the protein supplied (Horwitt, '55). Estimations of how much tryptophan is convertible to niacin and its derivatives depend largely upon the study of the three main excretory products of niacin-tryptophan metabolism, N^1-Me, the 6-pyridone of N^1-Me, and quinolinic acid. Analysis for 6-pyr can give useful information when relatively large amounts of N^1-Me are excreted, but when the levels

of N^1-Me approach the critical range in which some doubts may arise about the niacin adequacy of the diet, the amounts of the pyridone found in urine, using the analytical methods now available, are negligible. Quinolinic acid excretion does not appear to be as sensitive an index as N^1-Me to decreases in the niacin equivalence of the diet. The average quinolinic acid excretion for the subjects on the U-100 and R-100 diets declined to about 2.9 mg per day in the second month and did not show a significant decrease for the remainer of the experiment (see discussion of quinolinic acid data). It therefore appears expedient to judge niacin equivalence in terms of N^1-Me excretion as this compound is the most sensitive index at low levels of niacin-tryptophan intake and its determination is less subject to analytical errors than that available for either 6-pyr or quinolinic acid.

If one takes the average of N^1-Me excretion of the combined U-100 and R-100 groups and compares these data with those from the subjects in the NR-100 and TR-100 groups at 16, 24 and 36 weeks, one obtains a niacin:tryptophan efficiency of 1:86, 1:46 and 1:52, respectively. An example of the calculation used to obtain these ratios follows: taking the data from the 36th week (see fig. 2) the average daily N^1-Me excretions of the combined U-100 and R-100 groups, the NR-100 group and the TR-100 group are 0.92, 4.26 and 1.58, respectively. The average increase in N^1-Me excretion produced by feeding 10 mg of niacin is 4.26 mg minus 0.92 mg or 3.34 mg or 0.334 per milligram of niacin. Similarly, the average increase, above that obtained with the basal diet by feeding an additional 100 mg of tryptophan is 1.58 mg minus 0.92 mg or 0.66 mg, or 0.0066 mg N^1-Me per milligram of tryptophan. Therefore, during the 36th week, each milligram of niacin produced as much N^1-Me as 52 mg of tryptophan.

A similar calculation made from the average data obtained during the 46th week on those subjects who had not received lactalbumin gave, for three subjects in the NR-100 group, 4 from the TR-100 and 6 from the UR-100 group, a ratio of

1:65. In subsequent weeks, there were too few subjects not receiving extra protein to warrant such calculations.

From such data as presented above, a factor of 1:60 has been chosen to designate the relative efficacy of tryptophan in substituting for niacin. It is the opinion of the senior author, after attempting to analyze the data from each subject that, though there are large individual variations, a factor of 1:60 is a logical starting point for future studies. Past experience with "load tests" with other vitamins has shown that the proportions of a vitamin or its products excreted after ingestion are dependent upon the nutritional state of the subject being tested. Thus, the administration of a vitamin supplement equivalent to one day's requirement would, if given to a deficient subject, produce little urinary excretion of the given vitamin or its products. Therefore, since the calculations above compare groups in different stages of niacin saturation, one can predict that a larger proportion of the administered niacin would be excreted by the NR group than by the TR group which would produce an apparent ratio that would make tryptophan seem less effective than niacin. In other words, the 1:60 ratio includes a considerable margin of safety; less tryptophan than suggested by the ratio of 1:60 would probably be needed to replace each milligram of niacin in the non-growing adult.

The data discussed in the section on the effects of lactalbumin supplementation on N^1-Me excretion, though meager, do not show any marked differences between the effects of tryptophan fed as a component of lactalbumin and of L-tryptophan fed as an isolated amino acid.

DISCUSSION OF NIACIN RATIOS

Analysis of the data reported should take into account the results of the Tulane studies (Goldsmith et al., '52) some of which were conducted at the same time as the Elgin project. These workers produced niacin deficiency in three subjects in 50 days on a corn diet which contained about 4.7 mg of niacin and 190 mg of tryptophan. On the other

hand, no clinical signs of pellagra were noted in 95 days on a "wheat" diet which contained 5.7 mg of niacin and 230 mg of tryptophan. The Elgin project might, in part, be considered an extension of the Tulane "wheat" study as the 5.8 mg of nicotinic acid and 265 mg of tryptophan were given for as long as 87 weeks, and although some of the N^1-Me excretions obtained in Elgin were as low as the lowest obtained in subjects who showed clinical pellagra in the Tulane study on corn, the rate of decrease in N^1-Me excretion was slower in the Elgin study.

As a consequence of impressions gained from much research which preceded the establishment of the fact that tryptophan in dietary protein can replace niacin in the diet, the literature on niacin requirements has become overly complicated. If one restricts the discussion of niacin-tryptophan relationships to that part of the pellagra syndrome which is ameliorated by feeding niacin or tryptophan or both, one can obtain a practical viewpoint of the problem.

It is not reasonable to assume that an inflexible relationship exists between niacin and tryptophan which could be maintained through all genetic and dietary variation (including loss in preparation or differences in availability) but in this respect the requirements for niacin are not different from the requirements for other vitamins. The attention focused on the relationship between corn consumption and pellagra has been so intensive that when certain population groups which subsisted almost entirely on corn were found to be free of pellagra, reasons other than variations in intake of niacin and tryptophan have been sought to explain the observations.

Table 8 attempts to correlate the pellagragenic potential of several diets by assuming that 60 mg of tryptophan is equivalent to 1 mg of niacin. Except for the estimations of the "Goldberger" diet, the figures for niacin and tryptophan in table 8 are based on analyses of the cooked diet as served. Approximations of the tryptophan content of the "Goldberger" diet are hazardous to estimate despite the use of the excellent data published by Frazier and Friedemann ('46).

TABLE 8

Niacin ratios of experimental diets

DIET	CALORIES	NIACIN	TRYPTOPHAN	NIACIN EQUIVALENTS	NIACIN RATIO (niacin equivalents per 1000 Cal.) [1]	PROPORTION WITH PELLAGRA SYMPTOMS [2]
		mg	*mg*			
Tulane "corn" [3]	1700–2100 [1]	4.4–4.6	177–195	7.4–7.8	(3.7–3.9)	3/3
Tulane "wheat" [4]	1600–1900	4.2–4.7	177–193	7.3–7.8	(3.7–3.9)	2/3
Goldberger [5]	3000	6.7	330	12.2	4.1	6/11
Tulane "wheat" [4]	1750	4.9	200	8.2	(4.1)	1/2
Elgin (90%)	2070	5.2	238	9.2	4.4	0/1
Elgin (100%)	2300	5.8	265	10.2	4.4	0/11
Elgin (110%)	2530	6.4	292	11.3	4.4	0/1
Elgin (120%)	2760	7.0	318	12.3	4.4	0/2
Tulane "corn" + niacin [6]	1970	6.7	190	9.9	4.9	0/1

[1] Diets providing less than 2000 calories are arbitrarily calculated as containing 2000 calories on assumption that there is a minimum basal requirement for niacin-tryptophan of approximately 8.8 niacin equivalents and that increased needs for greater caloric consumption are measurable above this level as a multiple of 1000 calories (see text, pp. 35, 36).

[2] Calculation of data presented in a recent summary of the Tulane experiments (Goldsmith, personal communication) shows that symptoms of pellagra were obtained in 13 out of 15 individual subject trials when 8.7, or less, niacin equivalents were fed. Of these 15 subjects, symptoms were obtained in three out of 5 on wheat diets, 4 on U. S. corn, three on lime-treated Guatemalan corn, and two on untreated Guatemalan corn.

[3] Goldsmith et al. ('52).

[4] Goldsmith, personal communication.

[5] Calculated from Frazier and Friedemann ('46).

[6] Same diet as "Tulane corn" above, plus 2 mg of niacinamide per day.

Ten per cent was deducted for cooking loss (see table 1) from the estimated tryptophan content of the components of the "Goldberger" diet; the resulting calculated figure of 330 mg of tryptophan per 3000 calories for a diet high in corn products is not inconsistent with recent analytical experience.

It has been customary to calculate niacin requirements in terms of caloric intake (N.R.C. Recommended Dietary Allowances, '53). This procedure was originated before tryptophan was known as a niacin precursor, more because niacin requirements were estimated as 10 times the thiamine needs, than because of any special research to prove the relationship of niacin with caloric intake.

The data in table 8 suggest that it may be as practical to calculate "niacin equivalents" as it is to estimate the riboflavin or thiamine content of a diet and that the onset of pellagra may be predictable from the available "niacin ratio" ("niacin equivalents" per 1000 calories) of a diet as consumed. The data suggest further that additional corn, *per se,* in a diet may not necessarily be pellagragenic since corn, itself, if given in sufficient amounts can provide adequate niacin equivalents, depending on it's source and preparation. (It should be emphasized that corn remains an inadequate source of good protein and that other nutritional inadequacies may become apparent if it forms too large a proportion of the diet.) It is recognized that other factors have been reported to affect niacin requirements on a corn-containing diet, but it now seems desirable to evaluate these factors in terms of the "niacin ratio."

In evaluating needs for tryptophan, which is required for many important functions other than conversion to niacin, one must consider this amino acid, like other protein components, as being required in amounts proportional to the size of the individual. To avoid the complications of making separate calculations for basal requirements and the requirements for the utilization of energy, it is suggested that 2000 calories be considered as a base line. In other words, in

estimating the "niacin ratio" of a diet, one never uses less than 2000 calories in the calculation even though the subject in question is consuming less than this amount. This eliminates the possibility of having an apparently adequate value of this ratio in a subject consuming only a small part of a well-balanced diet. In effect, the amount adequate for 2000 calories would be the minimum basal requirement for the adult.

An alternate calculation of the minimum niacin-tryptophan requirements of man might be made by subtracting a minimum figure for tryptophan requirements (Rose, '49) for maintenance (about 150 mg or 2.5 niacin equivalents) and then estimating the remaining niacin equivalents. However, since less than 200 mg of tryptophan are found only in experimental diets or in areas of severe starvation, no practical point is served by treating the maintenance requirement separately. The niacin ratio, if used as suggested, tends to correct for differences in the size of the individual, as size, protein and basal caloric needs are all related although it is recognized that the tryptophan needs are only indirectly related to caloric intake.

Table 9 lists the niacin ratios of some representative foods to show how relatively unimportant can be the niacin content of good sources of tryptophan. This table also demonstrates how corn itself can vary. To obtain the data on corn, calculations were based on the niacin and tryptophan contents of 23 different varieties of Guatemalan corn as reported by Aguirre et al. ('53). The 4 Guatemalan corns chosen in table 9 are those which contained the lowest niacin, the lowest tryptophan, the highest niacin and the highest tryptophan contents, respectively. In order to estimate the caloric content of these corns, the results published by Bressani et al. ('53) were calculated from their data on fat, nitrogen, crude fiber and ash. The average niacin ratio of the Guatemalan corns is about 6.7 and pellagra is relatively uncommon in Central America where it is claimed that the average Indian consumes about 500 gm of corn per day. This is considered to provide

about 80% of the total calories ingested. The average North American maize does not appear to be significantly different from Central American maize in tryptophan-niacin content (Teas and Newton, '51). It is of interest to note that white flour, which, when substituted for corn in an experimental diet (Goldsmith et al., '52) produced only a slightly greater excretion of N^1-Me, has a niacin ratio of 7.4. Salt pork and

TABLE 9

Niacin ratios of representative foods[1]

FOOD	NIACIN	TRYPTOPHAN	NIACIN RATIO
	mg/1000 Cal.	*mg/1000 Cal.*	
Cow's milk	1.21[2]	673[2]	12.4
Human milk	2.46[2]	443[2]	9.84
Beef, round	24.7	1280	46.0
Whole eggs	0.60	1150	19.8
Salt pork	1.15	61	2.17
Wheat flour	2.48	297	7.43
Corn grits[4]	1.83	70	3.00
Guatemalan corn[5]:			
lowest niacin	3.37	132	5.57
lowest tryptophan	5.07	35	5.65
highest niacin	6.94	101	8.62
highest tryptophan	4.50	158	7.13

[1] Block and Bolling ('51) used for tryptophan data in this table unless otherwise noted.

[2] Macy, Kelley and Sloan ('50).

[3] Williamson ('44).

[4] Calculated from data of Krehl et al. ('46).

[5] See text.

corn grits, common constituents of pellagragenic diets in this country, have low "niacin ratios."

Although corn products have the lowest "niacin ratio" of any common high-protein foods (table 9) and pellagra is usually associated with corn consumption, corn, itself, may not be a direct cause of pellagra. In this country, pellagragenic diets are mixtures of unenriched corn products and other foods. These other foods are high in caloric content but very low in niacin equivalents. From the data in tables 8 and 9 one might conclude that if one-third to one-half the corn in

the Guatemalan diet were to be replaced by "civilized" forms of carbohydrate, such as corn syrup, corn starch or sucrose, pellagra would be common in that country.

The statements made, herein, are not intended to eliminate the possibility that some corn products may have such low "niacin ratios," or such low niacin-tryptophan availability, that their consumption, exclusively, would promote pellagra. Rather it is an attempt to focus more attention on a simple relationship of the niacin-tryptophan content of the diet in terms of total calories consumed. Justification for using calories as a base line, rather than body weight, may be obtained from comparisons of the Goldberger, Tulane and Elgin diets and from experience with animals where niacin and thiamine needs were directly related. The Goldberger prison diet (Goldberger and Wheeler, '20) provided relatively high amounts of niacin and tryptophan but the caloric intake was also high. The prisoners had work to perform, whereas the Tulane and Elgin subjects had a minimum amount of activity. Krehl et al. ('46) have pointed out that their estimations of the tryptophan requirement of the rat was considerably less than the level of 0.2% suggested by Rose ('37) who used a diet relatively high in fat and calories.

The data which suggest that L-lysine supplementation (1 gm per day) had a deleterious effect on the formation of N^1-Me reinforce reports (Koeppe and Henderson, '55; Hankes et al., '49) that amino acids in the diet must be properly balanced.

When the diet of the TR group (about 9% protein) was fed to weanling rats (Horwitt, '53) their growth rate averaged less than 1.3 gm per day and fatty livers were produced. Adult men were reasonably well maintained on the same diet although some evidence of liver dysfunction was obtained. The addition of lactalbumin to this diet produced normal rats with a growth rate of 4.3 gm per day. These observations should be considered in the light of the increased protein requirements for rapid growth, and the high frequency of kwashiorkor in Guatemalan children on corn diets which do not produce pellagra.

SUMMARY

Controlled feeding of a diet which provided only 5.8 mg of niacin and 265 mg of tryptophan per 2300 calories to 15 subjects for from 38 to 87 weeks produced no clinical evidence of pellagra. Data from studies of niacin metabolites excreted in the urine of these subjects were compared with results obtained at the same time from 15 other subjects who received the experimental diet plus controlled amounts of tryptophan, niacin and lactalbumin. Another group of 9 subjects who ate the regular hospital diet, were observed simultaneously.

Comparisons of the levels of N^1-methylnicotinamide excreted at different levels of niacin and tryptophan intake showed that approximately 60 mg of tryptophan are equivalent to 1 mg of niacin. It is suggested that one "niacin equivalent" be considered as equal to either of these amounts. To simplify discussions of food values, the term "niacin ratio" is suggested as the number of niacin equivalents per 1000 calories. The niacin ratio of representative foods and of some experimental diets which have promoted pellagra are presented and compared to show the predictability of pellagragenic diets.

From a comparison of the data obtained in this study with previously published information from other sources, it is estimated that the base line level for the development of pellagra is 8.8 niacin equivalents for the first 2000 calories, or part thereof, plus an additional 0.44 equivalents for each 100 calories above 2000.

Tryptophan in lactalbumin appears to be as readily available for conversion to niacin derivatives as is L-tryptophan, itself.

The nitrogen excretion data reported emphasize the length of time which it may take to achieve a steady state of urinary nitrogen excretion (24 weeks) after a major change has been made in the protein intake. This observation is consistent with experience on all previous Elgin projects.

Data on urinary excretions of quinolinic acid could be only roughly related to the amounts of niacin and tryptophan in the diet; the results were not so consistent or as reliable as similar data obtained on N^1-methylnicotinamide excretion.

Urinary excretions of niacin and tryptophan were not directly related to the usual levels at which these were present in the diet.

Data pertaining to the possibility that lysine or methionine added as supplements may inhibit the formation of niacin metabolites are presented and discussed.

While attempting to correlate the effects of riboflavin deficiency on "pellagragenic" diets the following observations were made; (1) That a diet low in niacin and tryptophan did not cause any noticeable alterations in the previously reported course of ariboflavinosis; (2) that supplementation with vitamin B_{12} produced no changes in either the development or repair of ariboflavinosis and (3) confirmed a previous report that scrotal dermatitis was a consistent observation in ariboflavinosis in man.

The withdrawal of folic acid, calcium pantothenate, pyridoxine, and vitamin B_{12} as supplements to the basal diet of 5 subjects for a period of 25 weeks had no noticeable effect on the subjects involved. One may assume that either the basal diet was not grossly deficient in these vitamins or that the previous 62 weeks of supplementation had created a large reserve.

The level of excretion of N^1-methylnicotinamide has been confirmed as an indicator of the ability of the organism to extract niacin derivatives from the diet and in this manner may prove to be a useful index of niacin-tryptophan availability.

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LITERATURE CITED

AGRICULTURAL HANDBOOK No. 8 1950 Composition of Foods, U. S. Dept. of Agriculture.

AGUIRRE, F., R. BRESSANI AND N. S. SCRIMSHAW 1953 The nutritive value of Central American corns. III. Tryptophan, niacin, thiamine and riboflavin content of twenty-three varieties in Guatemala. Food Research, *18:* 273.

BLOCK, R. J., AND D. BOLLING 1951 Amino acid composition of proteins and foods. 2nd Ed. C. C Thomas, Springfield, Ill.

BONSNES, R. W., AND H. H. TAUSSKY 1945 On the colorimetric determination of creatinine by the Jaffe reaction. J. Biol. Chem., *158:* 581.

BRESSANI, R., G. ARROYAVE AND N. S. SCRIMSHAW 1953 Nutritive value of Central American corns. I. Nitrogen, ether extract, crude fiber and minerals of twenty-four varieties of corn. Food Research, *18:* 261.

BREWER, G. E. F., W. S. BROWN, C. C. HARVEY AND M. K. HORWITT 1947 Variations of the individual blood plasma amino acid nitrogen level. J. Biol. Chem., *168:* 145.

BURCH, H. B., O. A. BESSEY AND O. H. LOWRY 1948 Fluorimetric measurements of riboflavin and its natural derivatives in small quantities of blood serum and cells. Ibid., *175:* 457.

CARPENTER, K. J., AND E. KODICEK 1950 The fluorimetric estimation of N^1-methylnicotinamide and its differentiation from coenzyme I. Biochem. J., *46:* 421.

DIETRICK, J. E., G. D. WHEDON AND E. SHORR 1948 Effect of immobilization upon various metabolic and physiological functions of normal men. Am. J. Med., *4:* 3.

ELVEHJEM, C. A. 1948 Tryptophan and niacin relations and their implications to human nutrition. J. Am. Dietet. Assn., *24:* 653.

FRAZIER, E. I., AND T. E. FRIEDEMANN 1946 Pellagra, a study in human nutrition. Quart. Bull. Northwestern Univ. Med. School, *20:* 24.

GOLDBERGER, J., AND G. A. WHEELER 1920 Experimental production of pellagra in human subjects. U. S. Pub. Health Service, Hygiene Lab. Bull., *120.*

GOLDSMITH, G. A., H. P. SARETT, U. D. REGISTER AND J. GIBBENS 1952 Studies of niacin requirements in man. I. Experimental pellagra in subjects on corn diets low in niacin and tryptophan. J. Clin. Invest., *31:* 533.

GREENE, R. D., AND A. BLACK 1944 The microbiological assay of tryptophan in proteins and foods. J. Biol. Chem., *155:* 1.

GREENHUT, I. T., B. S. SCHWEIGERT AND C. A. ELVEHJEM 1946 Hydrolysis procedures for the determination of tryptophan in proteins and foodstuffs by the microbiological procedure. Ibid., *165:* 325.

HANKES, L. V., L. M. HENDERSON AND C. A. ELVEHJEM 1949 Effect of cystine and threonine on the growth of rats receiving tryptophan-deficient rations. Ibid., *180:* 1027.

HARVEY, C. C., AND M. K. HORWITT 1949 Excretion of essential amino acid by men on a controlled protein intake. Ibid., *178:* 953.

HENDERSON, L. M. 1949 Quinolinic acid metabolism. II. Replacement of nicotinic acid for the growth of the rat and neurospora. Ibid., *181:* 677.

HENDERSON, L. M., AND E. E. SNELL 1948 A uniform medium for determination of amino acids with various micro-organisms. Ibid., *172:* 15.

HEPLER, O. E. 1949 Manual of Clinical Laboratory Methods. 4th Ed. Charles C Thomas, Springfield, Ill.

HIATT, H. H., W. S. ROTHWELL AND M. K. HORWITT 1952 Eosinopenia produced by ACTH in patients with schizophrenia. Proc. Soc. Exp. Biol. Med., *79:* 707.

HILLS, O. W., E. LIEBERT, D. L. STEINBERG AND M. K. HORWITT 1951 Clinical aspects of dietary depletion of riboflavin. Arch. Int. Med., *87:* 682.

HOLMAN, W. I. M., AND D. J. DE LANGE 1949 Methods for the determination of N-methyl-2-pyridone-3-carboxylamide in human urine. Biochem. J., *45:* 559.

HORWITT, M. K. 1953 Report of Elgin Project No. 3 with emphasis on liver dysfunction. Current Research on Vitamins in Trophology, Nutrition Symposium Series, *7:* 67. National Vitamin Foundation, Inc., N. Y.

———— 1955 Niacin-tryptophan relationships in the development of pellagra. Am. J. Clin. Nutrition, *3:* 244.

HORWITT, M. K., C. C. HARVEY, O. W. HILLS AND E. LIEBERT 1949a Correlation of urinary excretion of riboflavin with dietary intake and symptoms of ariboflavinosis. J. Nutrition, *41:* 247.

HORWITT, M. K., O. W. HILLS, C. C. HARVEY, E. LIEBERT AND D. L. STEINBERG 1949b Effects of dietary depletion of riboflavin. Ibid., *39:* 357.

HORWITT, M. K., AND O. KREISLER 1949 The determination of early thiamine-deficient states by estimation of blood lactic and pyruvic acids after glucose administration and exercise. Ibid., *37:* 411.

HORWITT, M. K., E. LIEBERT, O. KREISLER AND P. WITTMAN 1948 Investigations of human requirements for B-complex vitamins. National Academy of Sciences, Washington, D. C. Bull. Nat. Res. Council, No. 116.

HORWITT, M. K., W. S. ROTHWELL AND R. M. KARK 1953 Liver dysfunction in man and rats on experimental diets inadequate in protein. Fed. Proc., *12:* 417.

HORWITT, M. K., G. SAMPSON, O. W. HILLS AND D. L. STEINBERG 1949c Dietary management in a study of riboflavin requirements. J. Am. Dietet. Assn., *25:* 591.

KARK, R. M., M. K. HORWITT AND W. S. ROTHWELL 1953 Production and repair of experimental liver dysfunction in man by modification of dietary protein. J. Lab. Clin. Med., *42:* 823.

KOEPPE, O. J., AND L. M. HENDERSON 1955 Niacin-tryptophan deficiency resulting from imbalances in amino acid diets. J. Nutrition, *55:* 23.

KREHL, W. A., P. S. SARMA AND C. A. ELVEHJEM 1946 The effect of protein on nicotinic acid and tryptophan requirement of the growing rat. J. Biol. Chem., *162:* 403.

KREHL, W. A., P. S. SARMA, L. J. TEPLEY AND C. A. ELVEHJEM 1946 Factors affecting the dietary niacin and tryptophan requirement of the growing rat. J. Nutrition, *31:* 85.

MACY, I. G., H. KELLEY AND R. SLOAN 1950 The composition of milks. Bull. National Res. Council, No. 119.

NATIONAL RESEARCH COUNCIL 1953 Recommended Dietary Allowances, Food and Nutrition Board, National Academy of Sciences, Washington. Publication 302.

PORTER, C. C., I. CLARK AND R. H. SILBER 1947 The effect of pyridoxine analogues on tryptophan metabolism in the rat. J. Biol. Chem., *167:* 573.

———— 1948 The effect of B-vitamin deficiencies on tryptophan metabolism in the rat. Arch. Biochem., *18:* 339.

REDEMANN, C. E. 1939 A semimicro-Kjeldahl distillation apparatus. Ind. and Eng. Chem., Anal. Ed., *11:* 635.

ROBERTS, E. C., AND E. E. SNELL 1946 An improved medium for microbiological assays wiht *Lactobacillus casei.* J. Biol. Chem., *163:* 499.

ROSE, W. C. 1937 The nutritive significance of the amino acids and certain related compounds. Science, *86:* 298.

———— 1949 Amino acid requirements of man. Fed. Proc., *8:* 553.

ROSEN, F., W. A. PERLZWEIG AND I. G. LEDER 1949 Fluorometric method for determination of the 6-pyridone of N^1-methylnicotinamide in urine. J. Biol. Chem., *179:* 157.

SARETT, H. P. 1951 Quinolinic acid excretion and metabolism in man. Ibid., *193:* 627.

SARETT, H. P., AND G. GOLDSMITH 1950 Metabolism of L- and DL-tryptophan in normal man and in pellagrins. Ibid., *182:* 679.

SEBRELL, W. H., AND R. E. BUTLER 1938 Riboflavin deficiency in man. Pub. Health Rep., *53:* 2282.

SMITH, D. T. 1942 In Biological Action of Vitamins. E. A. Evans, Jr., Ed. p. 84, U. of Chicago Press, Chicago, Ill.

SMITH, D. T., AND J. M. RUFFIN 1937 Effect of sunlight on clinical manifestations of pellagra. Arch. Int. Med., *59:* 631.

SNELL, E. E., AND L. D. WRIGHT 1941 A microbiological method for the determination of nicotinic acid. J. Biol. Chem., *139:* 675.

STEELE, B. F., H. E. SAUBERLICH, M. S. REYNOLDS AND C. A. BAUMANN 1949 Media for *Leuconostoc mesenteroides* P-60 and *Leuconostoc citrovorum* 8081. Ibid., *177:* 533.

TEAS, H. J., AND A. C. NEWTON 1951 Tryptophan, niacin and indolacetic acid in several endosperm mutants and standard lines of maize. Plant Physiol., *26:* 494.

THORN, G. W., P. H. FORSHAM, F. T. G. PRUNTY AND A. G. HILLS 1948 A test for adrenal cortical insufficiency. J. Am. Med. Assn., *137:* 1005.

WILLIAMSON, M. B. 1944 Amino acid composition of human milk protein. J. Biol. Chem., *156:* 47.

WOOLLEY, D. W. 1946 Occurrence of a "pellagragenic" agent in corn. Ibid., *163:* 773.